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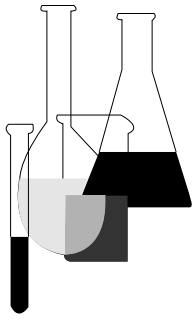
Health Effects Test Guidelines

OPPTS 870.5450
Rodent Dominant Lethal Assay



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading ''Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines.''

OPPTS 870.5450 Rodent dominant lethal assay.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are OPPT 40 CFR 798.5450 Rodent dominant Lethal assay and OECD 478 Genetic Toxicology: Rodent Dominant Lethal Assay.

(b) **Purpose.** Dominant lethal (DL) effects cause embryonic or fetal death. Induction of a dominant lethal event after exposure to a chemical substance indicates that the substance has affected germinal tissue of the test species. Dominant lethals are generally accepted to be the result of chromosomal damage (structural and numerical anomalies) but gene mutations and toxic effects cannot be excluded.

(c) **Definitions.** The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definition also applies to this test guideline.

Dominant lethal mutation is one occurring in a germ cell which does not cause dysfunction of the gamete, but which is lethal to the fertilized egg or developing embryo.

(d) **Reference substances.** These may include, but need not be limited to, triethylenemelamine, cyclophosphamide, or ethyl methanesulfonate.

(e) **Test method**—(1) **Principle.** Generally, male animals are exposed to the test substance and mated to untreated virgin females. The various germ cell stages can be tested separately by the use of sequential mating intervals. The females are sacrificed after an appropriate period of time and the contents of the uteri are examined to determine the numbers of implants and live and dead embryos. The calculation of the dominant lethal effect is based on comparison of the live implants per female in the treated group to the live implants per female in the control group. The increase of dead implants per female in the treated group over the dead implants per female in the control group reflects the post-implantation loss. The post-implantation loss is calculated by determining the ratio of dead to total implants from the treated group compared to the ratio of dead to total implants from the control group. Pre-implantation loss can be estimated on the basis of corpora lutea counts or by comparing the total implants per female in treated and control groups.

(2) **Description.** (i) Several treatment schedules are available. The most widely used requires single administration of the test substance.

Other treatment schedules, such as treatment on five consecutive days, may be used if justified by the investigator.

(ii) Individual males are mated sequentially to virgin females at appropriate intervals. The number of matings following treatment is governed by the treatment schedule and should ensure that germ cell maturation is adequately covered. Females are sacrificed in the second half of pregnancy and the uterine contents examined to determine the total number of implants and the number of live and dead embryos.

(3) **Animal selection**—(i) **Species.** Rats or mice are generally used as the test species. Strains with low background dominant lethality, high pregnancy frequency, and high implant numbers are recommended.

(ii) **Age.** Healthy, sexually mature animals should be used.

(iii) **Number.** An adequate number of animals should be used taking into account the spontaneous variation of the biological characteristics being evaluated. The number chosen should be based on the predetermined sensitivity of detection and power of significance. For example, in a typical experiment, the number of males in each group should be sufficient to provide between 30 and 50 pregnant females per mating interval.

(iv) **Assignment to groups.** Animals should be randomized and assigned to treatment and control groups.

(4) **Control groups**—(i) **Concurrent controls.** Generally concurrent positive and negative (vehicle) controls should be included in each experiment. When acceptable positive control results are available from experiments conducted recently (within the last 12 months) in the same laboratory, these results can be used instead of a concurrent positive control.

(ii) **Positive controls.** Positive control substances should be used at a dose which demonstrates the test sensitivity.

(5) **Test chemicals**—(i) **Vehicle.** When possible, test substances should be dissolved or suspended in isotonic saline or distilled water. Water-insoluble chemicals may be dissolved or suspended in appropriate vehicles. The vehicle used should neither interfere with the test chemical nor produce toxic effects. Fresh preparations of the test chemical should be employed.

(ii) **Dose levels.** Normally, three dose levels should be used. The highest dose should produce signs of toxicity (e.g., slightly reduced fertility and slightly reduced body weight). However, in an initial assessment of dominant lethality a single high dose may be sufficient. Nontoxic substances should be tested at 5 g/kg or, if this is not practicable, then as the highest dose attainable.

(iii) **Route of administration.** The usual routes of administration are oral or by IP injection. Other routes may be appropriate.

(f) **Test performance.** (1) Individual males are mated sequentially at appropriate predetermined intervals to one or two virgin females. Females should be left with the males for at least the duration of one estrus cycle or alternatively until mating has occurred as determined by the presence of sperm in the vagina or by the presence of a vaginal plug.

(2) The number of matings following treatment should be governed by the treatment schedule and should ensure that germ cell maturation is adequately covered.

(3) Females should be sacrificed in the second half of pregnancy and uterine contents examined to determine the number of implants and live and dead embryos. The ovaries may be examined to determine the number of corpora lutea.

(g) **Data and report**—(1) **Treatment of results.** Data should be tabulated to show the number of males, the number of pregnant females, and the number of nonpregnant females. Results of each mating, including the identity of each male and female, should be reported individually. For each female, the dose level and week of mating and the frequencies of live implants and of dead implants should be enumerated. If the data are recorded as early and late deaths, the tables should make that clear. If preplantation loss is estimated, it should be reported. Pre-implantation loss can be calculated as the difference between the number of corpora lutea and the number of implants or as a reduction in the average number of implants per female in comparison with control matings.

(2) **Statistical evaluation.** Data should be evaluated by appropriate statistical methods. Differences among animals within the control and treatment groups should be considered before making comparisons between treated and control groups.

(3) **Interpretation of results.** (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of dominant lethals. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of dominant lethals or a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) **Test evaluation.** (i) A positive DL assay suggests that under the test conditions the test substance may be genotoxic in the germ cells of the treated sex of the test species.

(ii) A negative result suggests that under the conditions of the test the test substance may not be genotoxic in the germ cells of the treated sex of the test species.

(5) **Test report.** In addition to the reporting recommendations as specified under 40 CFR part 792, subpart J, the following specific information should be reported:

(i) Species, strain, age, and weights of animals used, number of animals of each sex in experimental and control groups.

(ii) Test substance, vehicle used, dose levels and rationale for dosage selection, negative (vehicle) and positive controls, and experimental observations, including signs of toxicity.

(iii) Route and duration of exposure.

(iv) Mating schedule.

(v) Methods used to determine that mating has occurred (where applicable).

(vi) Criteria for scoring dominant lethals including the number of early and late embryonic deaths.

(vii) Dose-response relationship, if applicable.

(h) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Brewen, J.G. et al. Studies on chemically induced dominant lethality. I. The cytogenetic basis of MMS-induced dominant lethality in post-meiotic germ cells. *Mutation Research* 33:239–250 (1975).

(2) Ehling, U.H. et al. Standard protocol for the dominant lethal test on male mice. Set up by the Work Group Dominant lethal mutations of the ad hoc Committee Chemogenetics. *Archives of Toxicology* 39:173–185 (1978).